

1125722-0005

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Miller, et al.
Serial No. : 09/730,214 Examiner: M. Borin
Filed : December 5, 2000 Group Art Unit: 1631
For : METHOD AND SYSTEM FOR DESIGNING PROTEINS AND
PROTEIN BACKBONE CONFIGURATIONS

DECLARATION UNDER 37 CFR §1.132

I, Ned Wingreen, Ph.D. declare as follows:

I am a Professor in the Department of Molecular Biology at Princeton University in Princeton, New Jersey. Immediately prior to that, I was Senior Research Staff Member at NEC Laboratories America, Inc., Princeton, New Jersey, the Assignee of the above-identified application. Currently, I remain in the employ of NEC on a consultant basis. My curriculum vitae is attached as Exhibit A.

I am a coinventor of the subject matter of the above-identified patent application, and I participated in the February 23, 2005 Examiner interview. I am familiar with the Office Actions issued during the course of prosecution of this application and its offspring divisional applications. The studies set forth below were carried out by my collaborators and me.

The studies were performed to determine the effectiveness of the design method claimed in the above-referenced application

in identifying a novel, stable fold into which a novel sequence of amino acids can be configured.

Using the method of Miller et al., which is also the method disclosed and claimed in the present application, we identified a small, highly designable protein fold that did not appear as a stand-alone fold in the Protein Data Bank (PDB). [Miller, et al., Emergence of Highly Designable Protein-Backbone Conformations in an Off-Lattice Model. *Proteins* 47, 506-512 (2002), copy attached as Exhibit B.] For the particular analysis described below, the method consisted of the following steps: (1) generating backbone configurations of a preselected length n by complete enumeration using a set of three dihedral angle pairs, (2) assigning a sphere of radius 1.9 Å to the beta carbon position of each residue, (3) eliminating configurations for which any of these spheres overlapped, (4) evaluating the surface exposure of each sphere in each remaining configuration, and eliminating all but the ~10,000 configurations with the lowest total surface exposure, (5) normalizing the surface exposure of the spheres in each remaining configuration, (6) generating sequences of hydrophobicities h_i ($= 0$ or 1) of the same length as each of the remaining configurations, (7) determining for each sequence of hydrophobicities which of the remaining configurations was the ground state, (8) identifying those configurations which were ground states of the largest number of sequences of hydrophobicities, and (9) determining which of these configurations were novel, i.e. did not have a close match in the PDB.

By following the above steps we winnowed down the number of protein backbone configurations which merited consideration for design. First, the very large number of configurations generated from all possible combinations of the three dihedral angle pairs (3^n) was reduced to ~10,000 by considerations of self-overlap and compactness (see steps (1) to (4) above). Second, the remaining ~10,000 configurations were organized in a list starting from the configuration that was the ground state of the largest number of sequences. Configurations not falling near the top of this list (~ top 100) were considered unpromising for purposes of design. Finally, the top folds were tested for novelty by comparison with known protein backbone configurations in the PDB.

One fold identified in this way became our target for synthesis. In terms of secondary structural elements, the fold consisted of a beta strand followed by an alpha helix, followed by a second beta strand. The beta strands folded over the alpha helix creating a two-stranded beta sheet as shown in Fig. 1.

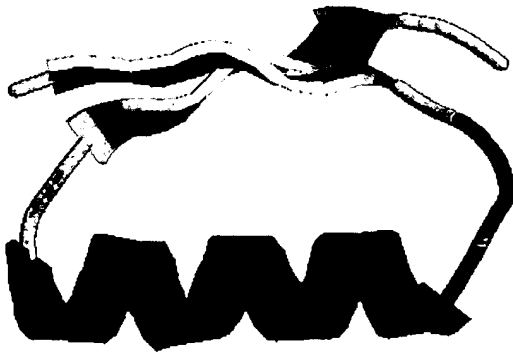


Figure 1. Ribbon diagram of beta-alpha-beta fold. The beta strands (yellow) form a beta sheet on top of the alpha helix (magenta).

A specific amino-acid sequence, of length 33 residues, was designed to adopt the desired backbone configuration, based on standard considerations of packing and solvent exposure. The designed sequence is KRRITLGGGEERIKKYREAFKNGNIEVTFQGG, using the single-letter code for amino acids. The predicted configuration of this sequence folded into the beta-alpha-beta configuration is shown in Fig. 2.

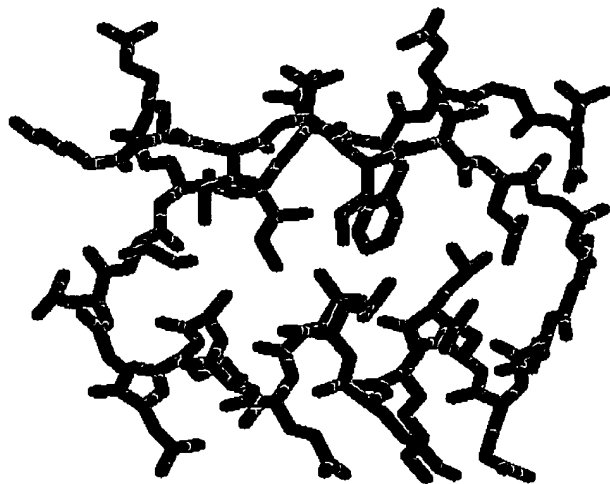


Figure 2. Predicted structure of sequence designed to adopt beta-alpha-beta fold. The detailed backbone and sidechain configurations for the 33-residue sequence are shown with nitrogens indicated in blue and oxygens in red.

The designed protein sequence of 33 residues was synthesized chemically and subjected to various analyses. First, the protein proved to be highly soluble in water, which allowed for standard biophysical tests. Specifically, the circular dichroism (CD) spectrum was obtained and analyzed (Fig. 3). The measured spectrum corresponds to an alpha helical content of 28% and a beta-strand content of 20%, which compare very favorably with the predicted values of 30% and 18%, respectively.

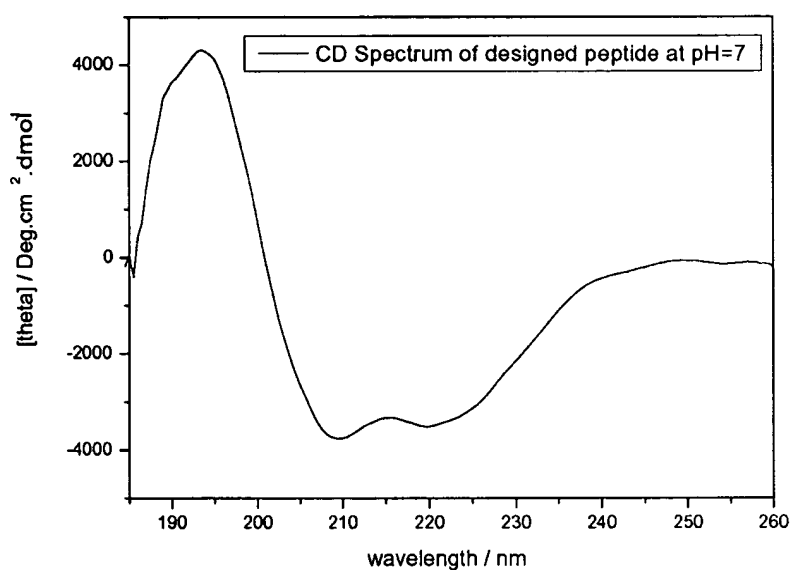


Figure 3. Measured circular dichroism (CD) spectrum of designed 33-residue sequence. The amplitudes of the characteristic features in the CD spectrum correspond to a folded structure consisting of 28% alpha helix and 20% beta strand.

The specific heat of thermal denaturation was also measured (Fig. 4) and found to be consistent with two-state folding, *i.e.* a direct transition between an ensemble of unfolded configurations and a single folded configuration with decreasing temperature.

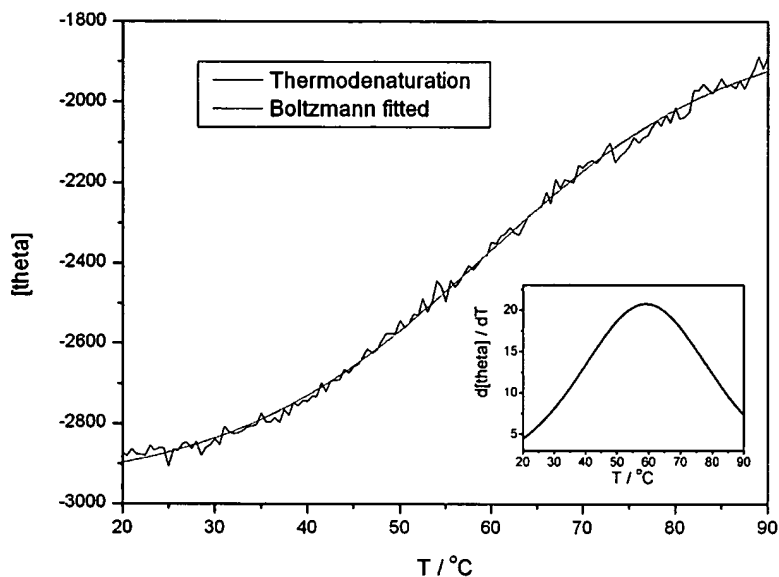


Figure 4. Measured specific heat of thermal denaturation of designed 33-residue sequence. The thermal denaturation curve (black) can be fitted extremely well by theoretical curve corresponding to two-state folding, consistent with the existence of a single well-folded configuration.

A 1D-NMR spectrum was obtained for the designed 33-residue sequence in solution (Fig. 5). The clear resolution of the peaks provided critical evidence that the designed sequence was indeed folding into a single unique structure. The peak widths proved somewhat too broad to allow reconstruction of the three-dimensional structure by 2D-NMR.

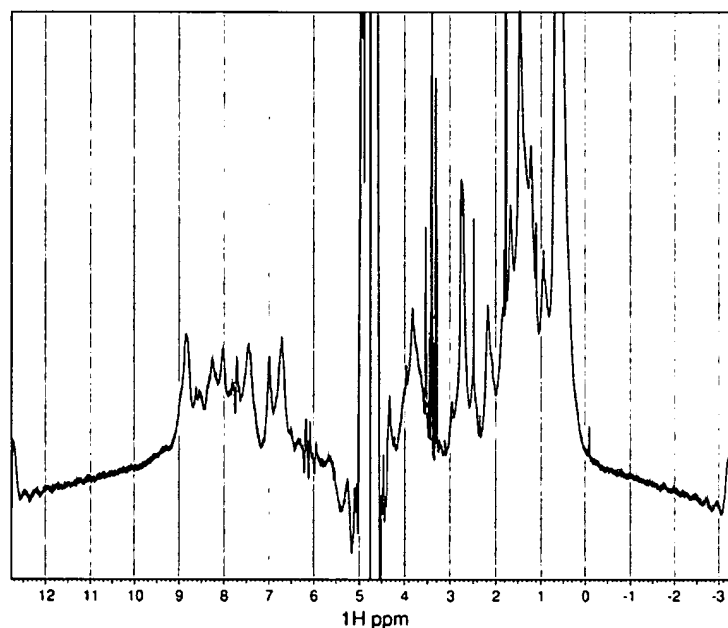


Figure 5. 1D-NMR spectrum of the designed 33-residue sequence. The multiple peaks are consistent with a single folded structure.

Together, the 1D-NMR spectrum and the CD spectrum provide strong evidence that not only is the designed sequence folding into a unique and stable structure, but that the unique structure is the target beta-alpha-beta fold.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that these statements were made with the knowledge that willful false

Serial No.: 09/730,214
Filed: December 5, 2000
Docket No. 1125722-0005

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 4/21/05

A handwritten signature in black ink, appearing to read 'Ned Wingreen', written over a horizontal line.

Ned Wingreen, Ph.D.

Ned S. Wingreen

Department of Molecular Biology – PRINCETON UNIVERSITY, Princeton, NJ 08544-1014

PHONE: 609-258-8476 FAX: 609-258-8616 EMAIL: wingreen@princeton.edu

EDUCATION

California Institute of Technology	Physics	B.S.	1984
Cornell University	Physics	M.S.	1988
Cornell University	Physics	Ph.D.	1989

Dissertation: Resonant Tunneling with Electron-Phonon Interaction.
Thesis adviser: Professor John W. Wilkins.

PROFESSIONAL EMPLOYMENT

9/84 – 5/89	Fannie and John Hertz Foundation Fellow, Lab of Atomic and Solid State Physics, Cornell University
5/89 – 9/89	Visiting Scientist, Weizmann Institute of Science, Israel
9/89 – 9/91	Postdoctoral Associate, Physics Department, MIT, Supervisor: Patrick A. Lee
9/91 – 3/99	Research Scientist, Physical Sciences Division, NEC Research Institute
4/99 – 10/02	Senior Research Scientist, Physical Sciences Division, NEC Research Institute
8/99 – 5/00	Sabbatical Visitor, University of California, Berkeley
11/02 – 1/04	Senior Research Staff Member, NEC Laboratories America, Inc.
2/04 – Present	Professor, Princeton University, Department of Molecular Biology

HONORS

Academic:

California Institute of Technology (1980-1984)
Presidential Scholar (1980)
Carnation Merit Scholarship (1982-1983)
Caltech Merit Scholarship (1983-1984)
Jack E. Froehlich Memorial Award (1983)
McKinney Prize in Literature (1984)

Cornell University (1984-1989)
Fannie and John Hertz Foundation Fellowship (1984-1989)

Professional:

Fellow of the American Physical Society

PATENTS

U.S. Patent No. 5,963,571, October 5, 1999, "Quantum-Dot Cascade Laser, Ned S. Wingreen
U.S. Patent No. 5,699,215, December 16, 1997, "Non-Magnetic Magnetoresistive Reading Head Using Corbino Structure," Stuart A. Solin, Ned S. Wingreen
U.S. Patent No. 5,692,003, November 25, 1997, "Quantum-Dot Cascade Laser," Ned S. Wingreen, Charles A. Stafford

PUBLICATIONS

- Morten Kloster, Chao Tang, Ned S. Wingreen,
Finding Regulatory Modules through Large-Scale Gene-Expression Data Analysis,
Bioinformatics. Oct 28; [Epub ahead of print] (2004).
- Naigong Zhang, Chen Zeng, Ned S. Wingreen,
Fast Accurate Evaluation of Protein Solvent Exposure,
Proteins, **57**: (3): pp. 565-76 (2004).
- Robert G. Endres, Thomas C. Schulthess, Ned S. Wingreen,
Toward an Atomistic Model for Predicting Transcription-Factor Binding Sites,
Proteins, **57**: (2): pp. 262-8. (2004).
- Ned S. Wingreen,
Quantum Many-Body Effects in a Single-Electron Transistor,
Science, **304**: (5675): pp. 1258-9 (2004).
- Kenji Hirose, Yigal Meir, Ned S. Wingreen,
Time-Dependent Density Functional Theory of Excitation Energies of Closed-Shell Quantum Dots,
Physica E **22**: (1-3): pp. 486-489 (2004).
- Derrick H. Lenz, Kenny C. Mok, Brendan N. Lilley, Rahul V. Kulkarni, Ned S. Wingreen, and Bonnie L. Bassler,
The Small RNA Chaperone Hfq and Multiple Small RNAs Control Quorum Sensing in *Vibrio harveyi* and *Vibrio cholerae*,
Cell **118**: pp. 69-82 (2004).
- Eldon G. Emberly, Ranjan Mukhopadhyay, Chao Tang, Ned S. Wingreen,
Flexibility of Beta-Sheets: Principal Component Analysis of Database Protein Structures,
Proteins: Structure, Function, and Genetics **55**: pp. 91-98 (2004).
- Kerwyn Casey Huang, Yigal Meir, Ned S. Wingreen,
Dynamic Structures in *Escherichia coli*: Spontaneous Formation of MinE Rings and MinD Polar Zones,
Proceedings of the National Academy of Science **100**:(22), pp. 12724-12728 (2003).
- Ned S. Wingreen, Hao Li, Chao Tang,
Designability and Thermal Stability of Protein Structures,
Polymer **45**: (2): pp. 699-705 (2004)
- Ranjan Mukhopadhyay, Eldon Emberly, Chao Tang, and Ned S. Wingreen,
Statistical Mechanics of RNA Folding: Importance of Alphabet Size,
Physical Review E **68**: pp. 041904(1-4) (2003).
- Ned S. Wingreen, Jonathan Miller, Edward C. Cox,
Scaling of Mutational Effects in Models for Pleiotropy,
Genetics **164**: pp. 1221-28 (2003).
- Kenji Hirose, Ned S. Wingreen,
Stabilization of Ground-State of Minimal Spin in Disordered Quantum Dots,
Physica E **18**: (1-3): pp. 79-80 (2003).
- Eldon Emberly, Ranjan Mukhopadhyay, Ned S. Wingreen, Chao Tang,
Flexibility of Alpha-Helices: Results of a Statistical Analysis of Database Protein Structures,
Journal of Molecular Biology **327**: pp. 229-37 (2003).

- Kenny C. Mok, Ned S. Wingreen, Bonnie L. Bassler,
***Vibrio harveyi* Quorum Sensing: A Coincidence Detector for Two Autoinducers Controls Gene Expression,**
EMBO Journal **22**: pp. 870-881 (2003).
- Kenji Hirose, Yigal Meir, Ned S. Wingreen,
Local Moment Formation in Quantum Point Contacts,
Physical Review Letters **90**:(2), pp. 026804(1-4) (2003).
- Eldon Emberly, Ned S. Wingreen, Chao Tang,
Designability of Alpha-Helical Proteins,
Proceedings of the National Academy of Science **99**: pp. 11163-8 (2002).
- Hao Li, Chao Tang, Ned S. Wingreen,
Designability of Protein Structures: A Lattice-Model Study using the Miyazawa-Jernigan Matrix,
Proteins: Structure, Function, and Genetics **49**: pp. 403-412 (2002).
- Jonathan Miller, Chen Zeng, Ned S. Wingreen, Chao Tang,
Emergence of Highly Designable Protein-Backbone Conformations in an Off-Lattice Model,
Proteins: Structure, Function, and Genetics **47**: pp. 506-512 (2002).
- Eldon Emberly, Jonathan Miller, Chen Zeng, Ned S. Wingreen, Chao Tang,
Identifying Proteins of High Designability Via Surface Exposure Patterns,
Proteins: Structure, Function, and Genetics **47**:(3), pp. 295-304 (2002).
- Yigal Meir, Kenji Hirose, Ned S. Wingreen,
Kondo Model for the 0.7 Anomaly in Transport through a Quantum Point Contact,
Physical Review Letters **89**:(19), pp. 196802(1-4) (2002).
- S. M. Cronenwett, H. J. Lynch, D. Goldhaber-Gordon, L. P. Kouwenhoven, C. M. Marcus, Kenji Hirose, Ned S. Wingreen, V. Umansky,
Low-Temperature Fate of the 0.7 Structure in a Point Contact: a Kondo-Like Correlated State in an Open System,
Physical Review Letters **88**:(22), pp. 226805(1-4) (2002).
- Kenji Hirose, Ned S. Wingreen,
Ground-State Energy and Spin in Disordered Quantum Dots,
Physical Review B **65**:(19), pp. 193305(1-4) (2002).
- Henry Cejtin, Jan Elder, Allan Gottlieb, Robert Helling, Hao Li, James Philbin, Chao Tang, Ned Wingreen,
Fast Tree Search For Enumeration of a Lattice Model of Protein Folding,
Journal of Chemical Physics **116**:(1), pp. 352-359, (2002).
- Robert Helling, Hao Li, Regis Melin, Jonathan Miller, Ned S. Wingreen, Chen Zeng, Chao Tang,
The Designability of Protein Structures,
Journal of Molecular Graphics and Modelling **19**:(1), pp. 157-167 (2001).
- Hao Li, Chao Tang, Ned S. Wingreen,
Designing Protein Structures,
Phase Transition and Self-Organization in Electronic and Molecular Networks, Phillips, J.C. (ed.) Kluwer pp. 441-445 (2001).
- Kenji Hirose, Shu-Shen Li, Ned S. Wingreen,
Mechanisms for Extra Conductance Plateaus in Quantum Wires,
Physical Review B **63**:(3), pp. 033315(1-4) (2001).

- Kenji Hirose, Fei Zhou, Ned S. Wingreen,
Density-Functional Theory of Spin-Polarized Disordered Quantum Dots,
Physical Review B **63**:(7), pp. 075301(1-5) (2001).
- Kenji Hirose, Fei Zhou, Ned S. Wingreen,
Spin-Density-Functional Theory of Clean and Disordered Quantum Dots,
Proceedings of the 25th International Conference on the Physics of Semiconductors-ICPS, Miura, N.(ed.), Springer, pp. 1349-1350 (2001).
- Kenji Hirose, Ned S. Wingreen,
Temperature-Dependent Suppression of Conductance in Quantum Wires: Anomalous Activation Energy from Pinning of the Band Edge,
Physical Review B **64**:(7), pp. 073305(1-4) (2001).
- Ned S. Wingreen,
The Kondo Effect in Novel Systems,
Materials Science and Engineering B **84**:, pp. 22-25 (2001).
- V. Madhavan, W. Chen, T. Jamneala, M.F. Crommie, Ned S. Wingreen,
Local Spectroscopy of a Kondo Impurity: Co on Au(111),
Physical Review B **64**:(16), pp. 165412(1-11) (2001).
- D.E. Grupp, T. Zhang, G.J. Dolan, Ned S. Wingreen,
Dynamical Offset Charges in Single-Electron Transistors,
Physical Review Letters **87**:(18), pp. 186805(1-4) (2001).
- Tairan Wang, Jonathan Miller, Chao Tang, Ned S. Wingreen, Ken A. Dill,
Symmetry and Designability for Lattice Protein Models,
Journal of Chemical Physics **113**:(18), pp. 8329-8336 (2000).
- Peter Nordlander, Ned S. Wingreen, Yigal Meir, David C. Langreth,
Kondo Physics in the Single Electron Transistor with ac Driving,
Physical Review B **61**:(3), pp. 2146-2150 (2000).
- Regis Melin, Hao Li, Ned S. Wingreen, Chao Tang,
Designability, Thermodynamic Stability, and Dynamics in Protein Folding: A Lattice Model Study,
Journal of Chemical Physics **111**0: pp. 1252-1262 (1999).
- Kenji Hirose, Ned S. Wingreen,
Spin-Density-Functional Theory of Circular and Elliptical Quantum Dots,
Physical Review B **59**:(7), pp. 4604-4607 (1999).
- Peter Nordlander, Michael Pustilnik, Yigal Meir, Ned S. Wingreen, David C. Langreth,
How Long Does it Take for the Kondo Effect to Develop? ,
Physical Review Letters **83**:(4), pp. 808-811 (1999).
- Igor E. Smolyarenko, Ned S. Wingreen,
Kondo Effect in Systems With Spin Disorder,
Physical Review B **60**:(13), pp. 9675-9689 (1999).
- K. Hirose, N. S. Wingreen,
Electronic Structure Calculations of Quantum Dots,
NEC Research and Development **40**:(4), pp. 419-423 (1999).
- C. Heide, R. J. Elliott, Ned S. Wingreen,
Spin-Polarized Tunnel Current in Magnetic-Layer Systems and its Relation to the Interlayer Exchange

Interaction,

Physical Review B **59**:(6), pp. 4287-4304 (1999).

- P. Jauho, Ned S. Wingreen,
Theory of Phase-Sensitive Measurement of Photon-Assisted Tunneling Through a Quantum Dot,
Physical Review B **58**:(15), pp. 9619-9622 (1998).
- N. S. Wingreen, B. L. Altshuler, Y. Meir,
Erratum: Comment on "2-Channel Kondo Scaling in Conductance Signals from 2-Level Tunneling Systems",
Physical Review Letters **81**:(19), pp. 4280 (1998).
- Naama Barkai, Mark D. Rose, Ned S. Wingreen,
Protease Helps Yeast Find Mating Partners,
Nature **396**:(6710), pp. 422-423 (1998).
- V. Madhavan, W. Chen, T. Jamneala, M.F. Crommie, Ned S. Wingreen,
Tunneling into a Single Magnetic Atom: Spectroscopic Evidence of the Kondo Resonance,
Science **280**: pp. 567-569 (1998).
- Hao Li, Chao Tang, Ned S. Wingreen,
Are Protein Folds Atypical?
Proceedings of the National Academy of Science **95**: pp. 4987-4990 (1998).
- L. P. Kouwenhoven, C. M. Marcus, P. L. McEuen, S. Tarucha, R. M. Westervelt, N. S. Wingreen,
Electron Transport in Quantum Dots,
Proceedings of the NATO Advanced Study Institute on Mesoscopic Electron Transport edited by L.L. Sohn, L.P. Kouwenhoven, and G. Schon (Kluwer Series E345) pp. 105-204 (1997).
- L. Aleiner, Ned S. Wingreen, Yigal Meir,
Dephasing and the Orthogonality Catastrophe in Tunneling Through a Quantum Dot: The "Which Path?" Interferometer,
Physical Review Letters **79**: pp. 3740-3743 (1997).
- Hao Li, Chao Tang, Ned S. Wingreen,
Nature of Driving Force for Protein Folding: A Result from Analyzing the Statistical Potential,
Physical Review Letters **79**: pp. 765-768 (1997).
- Ned S. Wingreen, Charles A. Stafford,
Quantum-Dot Cascade Laser: Proposal for an Ultralow-Threshold Semiconductor Laser,
IEEE Journal of Quantum Electronics **33**: pp. 1170-1173 (1997).
- Oded Agam, Ned S. Wingreen, Boris Altshuler, D. C. Ralph, M. Tinkham,
Chaos, Interactions, and Nonequilibrium Effects in the Tunneling Resonance Spectra of Ultrasmall Metallic Particles,
Physical Review Letters **78**: pp. 1956-1959 (1997).
- A. Yacoby, H.L. Stormer, Ned S. Wingreen, L. N. Pfeiffer, K. W. Baldwin, K. W. West,
Nonuniversal Conductance Quantization in Quantum Wires,
Physical Review Letters **77**: pp. 4612-4615 (1996).
- N.F. Schwabe, R.J. Elliott, Ned S. Wingreen,
The Ruderman-Kittel-Kasuya-Yosida (RKKY) Interaction Across a Tunneling Junction Out of Equilibrium,
Physical Review B **54**: pp. 12953-12968 (1996).

- Noam Sivan, Ned S. Wingreen,
The Single Impurity Anderson Model Out of Equilibrium,
Physical Review B **54**: pp. 11622-11629 (1996).
- Hao Li, Robert Helling, Chao Tang, Ned S. Wingreen,
Emergence of Preferred Structures in a Simple Model of Protein Folding
Science **273**: pp. 666-669 (1996).
- C. A. Stafford, Ned S. Wingreen,
Resonant Photon-assisted Tunneling Through a Double Quantum Dot: An Electron Pump from Spatial Rabi Oscillations,
Physical Review Letters **76**: pp. 1916-1919 (1996).
- Ned S. Wingreen, Eugen Schenfeld,
Size-speed Trade-off in Optical Switching Elements,
Applied Optics **34**: pp. 5907-5912 (1995).
- Ned S. Wingreen, Boris Altshuler, Yigal Meir,
Comment on "2-Channel Kondo Scaling in Conductance Signals from 2-Level Tunneling Systems,"
Physical Review Letters **75**: pp. 769 (1995).
- Yigal Meir, Ned S. Wingreen,
Spin-orbit Scattering and the Kondo Effect,
Physical Review B (Rapid Communications) **50**: pp. 4947-4950 (1994).
- Antti-Pekka Jauho, Ned S. Wingreen, Yigal Meir,
Time-dependent Transport in Interacting and Noninteracting Resonant-tunneling Systems,
Physical Review B **50**: pp. 5528-5544 (1994).
- Antti-Pekka Jauho, Ned S. Wingreen, Yigal Meir,
Time-dependent Transport in Mesoscopic Systems: General Formalism and Applications,
Semiconductor Science and Technology **9**: pp. 926-929 (1994).
- Ned S. Wingreen, Yigal Meir,
Anderson Model out of Equilibrium: Noncrossing-approximation Approach to Transport Through a Quantum Dot,
Physical Review B **49**: pp. 11040-11052 (1994).
- Mark Lee, Ned S. Wingreen, S. A. Solin, P. A. Wolff,
Giant Growth Axis Longitudinal Magnetoresistance from In-plane Conduction in Semiconductor Superlattices,
Solid State Communications **89**: pp. 687-691 (1994).
- A. Alan Middleton, Ned S. Wingreen,
Collective Transport in Arrays of Quantum Dots,
Physical Review Letters **71**: pp. 3198-3201 (1993).
- Jari M. Kinaret, Ned S. Wingreen,
Coulomb Blockade and Partially Transparent Tunneling Barriers in the Quantum Hall Regime,
Physical Review B **48**: pp. 11113-11119 (1993).
- Ned S. Wingreen, Antti-Pekka Jauho, Yigal Meir,
Time-dependent Transport Through a Mesoscopic Structure,
Physical Review B (Rapid Communications) **48**: pp. 8487-8490 (1993).

- P. L. McEuen, Ned S. Wingreen, E. B. Foxman, Jari Kinaret, U. Meirav, M. A. Kastner, Yigal Meir, **Coulomb Interactions and Energy-level Spectrum of a Small Electron Gas**, *Physica B* **189**: pp. 70-79 (1993).
- E. B. Foxman, P. L. McEuen, U. Meirav, Ned S. Wingreen, Yigal Meir, Paul A. Belk, N. R. Belk, M. A. Kastner, S. J. Wind, **Effects of Quantum Levels on Transport Through a Coulomb Island**, *Physical Review B (Rapid Communications)* **47**: pp. 10020-10023 (1993).
- Yigal Meir, Ned S. Wingreen, Patrick A. Lee, **Low-temperature Transport Through a Quantum Dot: The Anderson Model out of Equilibrium**, *Physical Review Letters* **70**: pp. 2601-2604 (1993).
- Jari M. Kinaret, Yigal Meir, Ned S. Wingreen, Patrick Lee, Xiao-Gang Wen, **Conductance Through a Quantum Dot in the Fractional Quantum Hall Regime**, *Physical Review B (Rapid Communications)* **45**: pp. 9489-9492 (1992).
- Jari M. Kinaret, Yigal Meir, Ned S. Wingreen, Patrick Lee, Xiao-Gang Wen, **Many-body Coherence Effects in Conduction Through a Quantum Dot in the Fractional Quantum Hall Regime**, *Physical Review B* **46**: pp. 4681-4689 (1992).
- Yigal Meir, Ned S. Wingreen, **Landauer Formula for the Current Through an Interacting Electron Region**, *Physical Review Letters* **68**: pp. 2512-2515 (1992).
- Jari M. Kinaret, Yigal Meir, Ned S. Wingreen, Patrick Lee, Xiao-Gang Wen, **Conductance Through a Quantum Dot in the Fractional Quantum Hall Regime**, *Physical Review B (Rapid Communications)* **45**: pp. 9489-9492 (1992).
- P. L. McEuen, E. B. Foxman, Jari Kinaret, U. Meirav, M. A. Kastner, Ned S. Wingreen, S. J. Wind, **Self-consistent Addition Spectrum of a Coulomb Island in the Quantum Hall Regime**, *Physical Review B (Rapid Communications)* **45**: pp. 11419-11422 (1992).
- P. L. McEuen, E.B. Foxman, U. Meirav, M.A. Kastner, Yigal Meir, Ned S. Wingreen, **Transport Spectroscopy of a Coulomb Island in the Quantum Hall Regime**, *Physical Review Letters* **66**: pp. 1926-1929 (1991).
- Yigal Meir, Ned S. Wingreen, Ora Entin-Wohlman, Boris L. Altshuler, **Spin-Orbit Scattering for Localized Electrons: Absence of Negative Magnetoconductance**, *Physical Review Letters* **66**: pp. 1517-1520 (1991).
- Yigal Meir, Ned S. Wingreen, Patrick A. Lee, **Transport Through a Strongly Interacting Electron System: Theory of Periodic Conductance Oscillations**, *Physical Review Letters* **66**: pp. 3048-3051 (1991).
- Ned S. Wingreen, **Rectification by Resonant Tunneling Diodes**, *Applied Physics Letters* **56**: pp. 253-255 (1990).
- Ned S. Wingreen, Karsten W. Jacobsen, John W. Wilkins, **Inelastic Scattering in Resonant Tunneling**, *Physical Review B* **40**: pp. 11834-11850 (1989).

- Ned S. Wingreen, Monique Combescot,
Electron-electron Scattering: Collision Integral and Relaxation Rate,
Physical Review B **40**: pp. 3191-3196 (1989).
- Ned S. Wingreen, Monique Combescot,
Ohm's Law for Hot Carriers: the Role of Carrier-carrier Scattering at High Fields,
Solid State Communications **70**: pp. 185-189 (1989).
- Ned S. Wingreen, Karsten W. Jacobsen, John W. Wilkins,
Resonant Tunneling with Electron-Phonon Interaction: An Exactly Solvable Model,
Physical Review Letters **61**: pp. 1396-1399 (1988).
- Ned S. Wingreen, Chris J. Stanton, John W. Wilkins,
**Electron-electron Scattering in Nondegenerate Semiconductors: Driving the Anisotropic Distribution
Toward a Displaced Maxwellian,**
Physical Review Letters **57**: pp. 1084-1087 (1986).

Emergence of Highly Designable Protein-Backbone Conformations in an Off-Lattice Model

Jonathan Miller, Chen Zeng, Ned S. Wingreen, and Chao Tang*

NEC Research Institute, Princeton, New Jersey

ABSTRACT Despite the variety of protein sizes, shapes, and backbone configurations found in nature, the design of novel protein folds remains an open problem. Within simple lattice models it has been shown that all structures are not equally suitable for design. Rather, certain structures are distinguished by unusually high designability: the number of amino acid sequences for which they represent the unique lowest energy state; sequences associated with such structures possess both robustness to mutation and thermodynamic stability. Here we report that highly designable backbone conformations also emerge in a realistic off-lattice model. The highly designable conformations of a chain of 23 amino acids are identified and found to be remarkably insensitive to model parameters. Although some of these conformations correspond closely to known natural protein folds, such as the zinc finger and the helix-turn-helix motifs, others do not resemble known folds and may be candidates for novel fold design. *Proteins* 2002;47:506–512.

© 2002 Wiley-Liss, Inc.

Key words: protein folds; off-lattice model; designability; protein design; evolution

INTRODUCTION

The de novo design of proteins—an object of enormous activity in recent years—has so far dealt primarily with the redesign of known protein folds.^{1–8} Two major accomplishments in the direction of designing a fold that is distinct from known natural folds are the synthesis of a right-handed coiled coil⁹ and the synthesis of a zinc finger without zinc.^{10–12} To challenge the best efforts of de novo design, nature offers roughly 1000 qualitatively distinct protein folds.¹³ Why has it proven difficult to design new protein folds? What program should we follow to achieve ab initio design of novel folds?

The principle of designability^{14–19} offers an answer to both these questions for simple lattice models. The designability of a structure is measured by the number of sequences that design it, that is, the number of sequences that have the given structure as their unique lowest energy conformation. Structures can differ vastly in their designability,¹⁴ and it has been shown that high designability entails other protein-like properties, such as mutational stability, thermodynamic stability,^{14,15} and fast folding kinetics.^{16,20} Design is hard in the sense that most structures have low designability and their associated

sequences lack these protein-like properties. For successful de novo design, one should first identify the few highly designable structures.

It is an open question whether designability applies to real proteins as it does to lattice polymers. Real protein structures have a degree of complexity that cannot be effectively represented within a simple lattice model. For example, on a lattice the angles between bonds differ from those naturally adopted in real proteins. In addition, although in a cubic-lattice model the cube minimizes surface area for a given volume and is perfectly packed, no counterpart of the perfect cube exists once the lattice is removed. For designability to guide practical design of new folds it must apply to realistic descriptions of protein structure.

In this article we report the computation of designability within an off-lattice model that incorporates angles favored by natural proteins, for protein chains of up to $N = 23$ amino acids. We find that the essential qualitative features of designability survive the transition from lattice model to off-lattice model. In particular, it remains true that a small fraction of compact structures are highly designable: these are nondegenerate ground states for an enormous number of amino acid sequences. Most structures, on the other hand, are ground states for few, if any, amino acid sequences. Furthermore, the sequences that fold into highly designable structures typically have enhanced thermodynamic stability—the energy of the nearest excited state is separated from the ground-state energy by an appreciable gap.

MODELS AND METHODS

The model we adopt is closely related to the off-lattice, m -state discrete-angle model introduced by Park and Levitt.²¹ Each configuration is defined by a sequence of C_α bonds of length 3.8 Å, and each pair of dihedral angles (ϕ , ψ) is restricted to one of only m alternatives; here we take $m = 3$. The set of m allowed angle pairs is chosen by fitting to the backbone coordinates of representative natural proteins,²¹ as discussed below. To suppress self-intersections of the chain, we augment the model by introducing a

C. Zeng's present address is Department of Physics, George Washington University, Washington, DC 20052.

*Correspondence to: Chao Tang, NEC Research Institute, 4 Independence Way, Princeton, NJ 08540. E-mail: tang@research.nj.nec.com

Received 23 October 2001; Accepted 7 January 2002

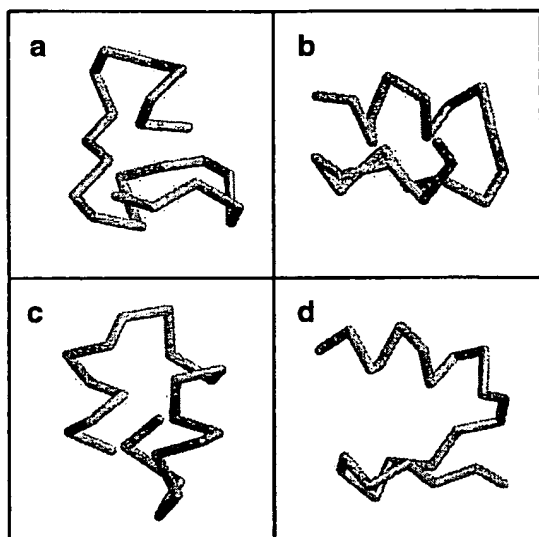


Fig. 1. a–c: Backbone configurations of 1st, 4th, and 15th most designable 23-mer structures. d: Backbone configuration of the zinc finger 1PSV,¹² truncated to 23 amino acids.

volume for the amino acid residues in the form of a sphere of radius r_p centered on C_p (the first carbon of the side-chain). The backbones of some configurations constructed in this fashion are shown in Fig. 1(a–c).

This off-lattice model incorporates properties of real polymers not well reproduced in simple lattice models. On the lattice, for example, allowed ground-state structures were limited to those maximally compact structures that fill the unique rectangle or box of minimum surface area. Off the lattice, every structure can be expected to have a distinct surface area. However, open or extended structures are not expected to be designable. We entertain as plausible ground-state structures only those with a surface area below some cutoff value A_c , which enters our computation as a parameter.*

Because a discrete angle set represents only a crude approximation to a continuum of angles, it is unrealistic to expect the surface area of a discrete-angle structure to faithfully reproduce the surface area of a structure built from more flexible angles. Importantly, using flexible angles would allow our more open structures (e.g., those just below the cutoff A_c) to contract and reduce their exposed surface areas. To achieve this equalizing effect of a continuum of angles within the limitations of a discrete-angle model, we normalize the vector of solvent-accessible surface areas $\mathbf{A} = (a_1, \dots, a_N)$, where a_i is the solvent-accessible surface area of the i -th residue, in such a way as to preserve the pattern of surface exposure along a chain.

A suitable procedure[†] is to normalize the vector \mathbf{A} for each structure by the total exposed surface area of that structure: $\bar{\mathbf{A}} = \mathbf{A}/\sum_i a_i = (\bar{a}_1, \dots, \bar{a}_N)$. This procedure treats all structures below the cutoff A_c as equally compact while preserving each structure's individual pattern of surface exposure along the chain.

As with real proteins, description and comparison of configurations off-lattice demands precision about what we mean by the term "structure." For example, a protein structure obtained by NMR represents an ensemble of configurations, no element of which necessarily provides a better fit to the data than any other. This ensemble presumably reproduces the temperature-induced fluctuations of a natural protein around its native state. On averaging over this ensemble for small stably folded polypeptides in the PDB database, one finds a typical center-of-mass root mean square (crms) of roughly 0.3–0.5 Å per residue. A similar range of crms can be inferred from the B values of protein crystals.²³ Accordingly, our off-lattice polymer configurations are grouped into clusters consisting of all configurations lying within a crms distance λ per residue of one another. Configurations within a cluster are to be thought of as variations of a single structure, and subsequently we will refer to clusters and structures interchangeably.

We define the designability of a structure as the sum of the designabilities of its included configurations. The designability of a configuration is simply the number of sequences with that configuration as a unique ground state.^{14,15} To evaluate the energy of a sequence on each configuration, we associate a hydrophobicity h_i with each amino acid of the sequence. In practice, we assign a hydrophobicity which is either 0 (Polar) or 1 (Hydrophobic) to each monomer to create an HP-sequence²⁴; that this is a reasonable simplification finds support in the work of Beasley and Hecht¹ [cf. Fig. 3(e) for the results of a more general choice]. The energy of a particular sequence folded into a particular configuration is obtained by taking the sum of the products of each amino acid's hydrophobicity h_i with its normalized surface exposure \bar{a}_i ,

$$E = \sum_i h_i \bar{a}_i \quad (1)$$

We numerically evaluate the energy of all HP-sequences for all configurations.

Except as indicated explicitly in the text, we choose discrete angles and the amino acid radius to optimize the fit to the backbone of the zinc-less synthetic zinc finger¹² 1PSV [Fig. 1(d)]. We find that there are many angle sets that fit the backbone of 1PSV almost equally well. For example, the crms per residue between 1PSV and the structure obtained from each of our 10 best angle sets varies from 0.844 to 0.913 Å. The angle set we use for most

*We evaluate the area of each C_p sphere accessible to a probe sphere of radius 1.4 Å, by the methods used in the program SERF,²² the slightly different values of surface area obtained by different methods do not in any way alter the outcome of the calculations.

[†]We have checked that certain alternative normalizations (e.g., normalizing by the total solvent-inaccessible surface area) do not alter the set of highly designable structures that emerge from our calculation. With no normalization, higher designability becomes closely correlated with lower solvent-accessible surface area.

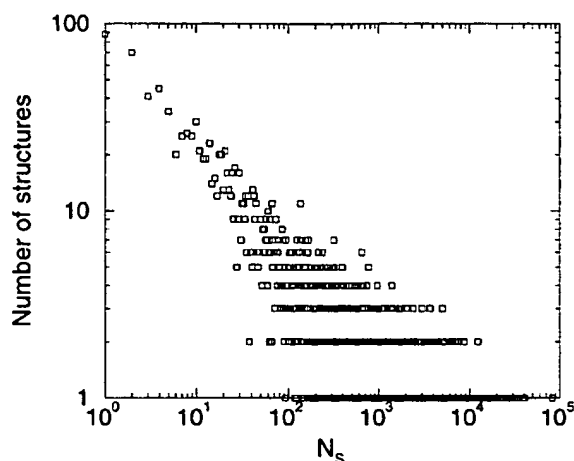


Fig. 2. Histogram of designabilities of 23-mer structures, using $r_\beta = 1.9$ Å. The surface area cutoff A_c is such that 10,000 configurations participate in the calculation, grouped into 4688 clusters with cluster radius $\lambda = 0.4$ Å.

of the calculations presented in this article is $(\phi, \psi) = (-95^\circ, 135^\circ)$, $(-75^\circ, -25^\circ)$, and $(-55^\circ, -55^\circ)$. The first pair lies in the β -region of the Ramachandran plot, and the other two pairs lie in the α -region. We take $r_\beta = 1.9$ Å, the radius above which the amino acids fit to the backbone of 1PSV would clash.

RESULTS

The designability of a structure denotes the number of distinct HP-sequences having that structure as their unique ground state. The distribution of designabilities for our model, displayed in Figure 2, reproduces a crucial feature first observed on the lattice: although most structures have very low designability, the trailing edge (or tail) of the distribution consists of a small number of structures of very high designability. Thus, designability distinguishes a small subset of structures from generic ones.

It turns out that the identities of these highly designable structures depend only weakly on the values of the parameters that enter our calculation: the surface area cutoff A_c , clustering radius λ , side-chain radius r_β , the set of allowed dihedral angles, and the range of amino acid hydrophobicities. More specifically, a significant fraction of structures identified as highly designable for one set of parameter values remains highly designable when these parameters are varied. We provide evidence for this important observation in the next five subsections.

Surface Area Cutoff

As discussed before, open structures are expected to exhibit low designability. We anticipate that the highly designable structures of interest to us will fall mainly within the class of compact structures; therefore, only these compact structures are needed in our calculation. The surface area cutoff A_c determines how compact a structure must be to qualify. We expect that, provided the choice of A_c is not too restrictive, its particular value ought not to be important.

A computationally practical choice of the surface-area cutoff eliminates most of the less compact configurations. A few of these might have proven highly designable if retained; however, our objective is not to find all highly designable structures, but only to identify some of them. Therefore, our major concern is not that we might incorrectly discard a few designable structures, but rather that we might produce false positives (structures that appear to be highly designable with a restrictive value of the cutoff but have low designability for a more relaxed cutoff). A larger cutoff admits previously disallowed configurations that “steal” some sequences from a configuration originally identified as highly designable, thereby reducing its designability.

In practice, as shown in Figure 3(a), highly designable structures tend to remain highly designable with increasing surface-area cutoff. For example, 9 of the 10 most designable structures remain within the 100 most designable even after the surface-area cutoff is relaxed sufficiently to admit a 10-fold increase in the number of participating structures.

Clustering Radius

As discussed in the previous section, structures whose backbones differ insignificantly from one another ought not to be considered distinct. This observation is embodied in our calculation by grouping into clusters those structures whose backbone configurations lie within a certain crms distance, λ , of one another. Varying the clustering radius, λ , leaves unchanged the set of configurations that participate in the calculation. For $\lambda \leq 0.1$ Å, nearly every cluster consists of a unique configuration. To exhibit the dependence of the most designable structures on λ , we fix a configuration and follow the designability of the cluster to which that configuration belongs, as a function of λ . As shown in Figure 3(b), the most designable structures remain roughly the same as λ is varied over a wide range.

Side-Chain Radius

Excluded volume is incorporated by means of a hard sphere of radius r_β centered on the β -carbon of each amino acid. Increasing the side-chain radius r_β eliminates some configurations because of steric clashes, whereas decreasing r_β admits previously ineligible configurations. Starting at $r_\beta = 1.9$ Å, we identify the most designable structures and then count the fraction of these structures that remain highly designable as r_β is reduced. As shown in Figure 3(c), the identities of the most designable structures are well preserved.

Set of Dihedral Angles

Next, we address to what extent an outcome depends on a particular choice of the discrete set of dihedral angles. A discrete set of angles cannot sample the structure space fully and so cannot “hit” all possible structures. On the other hand, we know that the designability of a structure depends on the local density of solvent-exposure vectors \vec{A} with highly designable structures occupying the lowest density regions.¹⁵ If the subset of structures sampled by a discrete set of angles reasonably preserves density in the

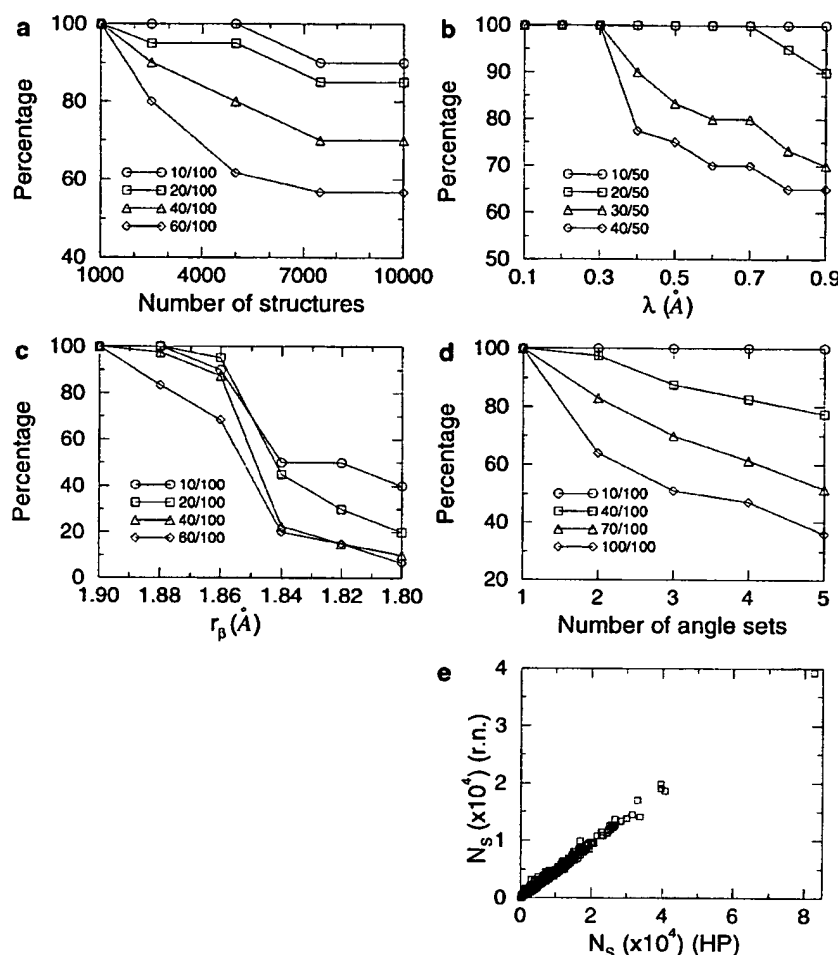


Fig. 3. Sensitivity to parameter changes of the most designable structures from Figure 2. **a:** Fraction of the 10, 20, 40, or 60 most designable structures that remain in the 100 most designable as the surface-area cutoff increases. The initial cutoff A_c is chosen so that only the 1000 most compact configurations participate and A_c increases until 10,000 configurations participate. **b:** Fraction of the 10, 20, 30, or 40 most designable structures that remain in the 50 most designable as the clustering radius λ is increased. The 5000 most compact configurations participate in the calculation and $r_b = 1.9$ Å. **c:** Fraction of the 10, 20, 40, or 60 most designable structures that remain in the 100 most designable as the side-chain radius r_b is changed. We have chosen the surface area cutoff so that 5000 structures participate in the designability calculation for $r_b = 1.9$ Å. If some configurations of the original most designable structures are not among the 5000 most compact configurations for some smaller r_b , we nevertheless retain them in the calculation. The clustering radius is $\lambda = 0.4$ Å. **d:** Fraction of the 10, 40, 70, or 100 most designable structures that remain in the 100 most designable as configurations from other angle sets are added. The values of the five angle sets are as follows set #1 = $(-95^\circ, 135^\circ), (-75^\circ, -25^\circ), (-55^\circ, -55^\circ)$; set #2 = $(-95^\circ, 135^\circ), (-85^\circ, -55^\circ), (-65^\circ, -25^\circ)$; set #3 = $(-105^\circ, 145^\circ), (-85^\circ, -15^\circ), (-75^\circ, -35^\circ)$; set #4 = $(-105^\circ, 145^\circ), (-85^\circ, -35^\circ), (-85^\circ, -5^\circ)$; set #5 = $(-105^\circ, 145^\circ), (-85^\circ, -35^\circ), (-85^\circ, -15^\circ)$. **e:** Designability of structures obtained from 4,000,000 randomly generated sequences of real numbers in $[0,1]$ versus designability from enumeration of HP-sequences. The 10000 most compact configurations participate in the calculation, $\lambda = 0.4$ Å, and $r_b = 1.9$ Å. (Note: the suppressed zeros in panels a, b, and d.)

space of structures, highly designable structures should remain highly designable as we improve our sampling of structure space.

To examine this possibility, we identify configurations generated by one angle set and follow their cluster designabilities as configurations from other angle sets are added. We take five different angle sets derived from fitting to 1PSV, and use the most compact configurations generated by each set. We calculate the designability of structures by

using configurations from, respectively, one, two, three, four, and finally all five sets. We observe in Figure 3(d) that the most designable structures in set #1 remain highly designable even as configurations from sets #2, #3, #4, and #5 are added. This result is maintained under permutation of the five sets. Apparently, any reasonable choice of angle set covers the structure space sufficiently well that highly designable structures can be identified with high probability.

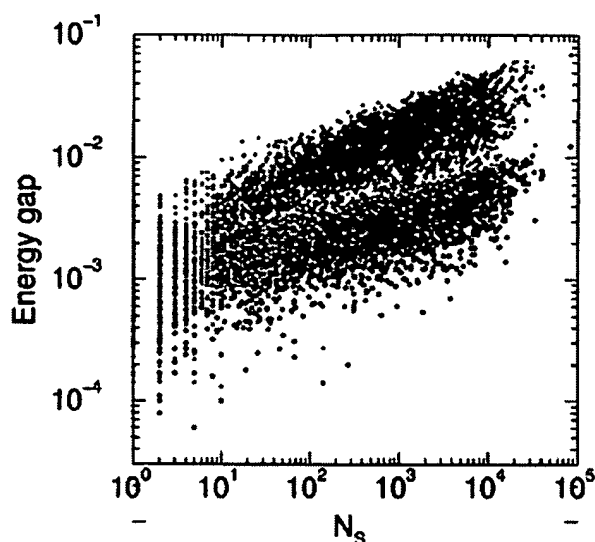


Fig. 4. Maximum energy gap (red dots) and average energy gap (black dots) for the HP-sequences that design a given structure, plotted versus structure designability. The 10,000 most compact configurations of the 23-mer participate in the calculation, with $\lambda = 0.4 \text{ \AA}$ and $r_p = 1.9 \text{ \AA}$.

HP Sequences

To check whether the identification of designable structures depends on our use of HP (binary) sequences of amino acids, we recalculate designabilities by using amino acids with continuous real-valued hydrophobicities. We randomly choose 4,000,000 sequences $\mathbf{h} = (h_1, \dots, h_N)$, where $h_i \in [0, 1]$, and evaluate their energy for all configurations using Eq. (1). In Figure 3(e) we plot the designability calculated this way against that from the enumeration of HP sequences. As the figure shows, the highly designable structures computed by these two alternative methods are nearly identical.

Parameter Independence

In the preceding five subsections we have shown that the parameters can sustain a considerable degree of variation without significantly changing the outcome of the designability calculation. The weak dependence of the set of highly designable structures on parameters is illustrated in Figure 3. Because the identity of the highly designable structures is robust to parameter variation, we now examine their potential as candidates for design.

Gap

In particular, a prerequisite for design is believed to be the presence of a large separation between the ground-state energy and the energy of the lowest excited state. For each structure, we have identified the HP-sequence that makes this gap the largest. The value of this largest gap is shown in Figure 4, as a function of the designability of the structure. To convert the vertical scale of Figure 4 to real energies, we observe that one unit of energy corresponds to a sequence of exclusively hydrophobic amino acids ($h_i = 1$) folded into one of our typical compact structures. Our

choice of surface area cutoff A_c guarantees that a typical compact configuration has around half of its maximal accessible surface exposed (about 25 \AA^2 per residue). A conservative estimate for the energy of exposed surface,²³ $20 \text{ cal/\AA}^2/\text{mol}$, then yields an energy on the order of 10 kcal/mol for a 23-mer. The highest gap energies achieved in Figure 4, of order 0.05 , therefore correspond to a gap of 0.5 kcal/mol , around $k_B T$ for room temperature. This gap is roughly the energy to promote one hydrophobic amino acid from core to surface. Also plotted is the average gap for all HP-sequences that design a structure. It is evident that high designability correlates strongly with a large gap.

DISCUSSION AND CONCLUSION

The principle of designability is that some structures are intrinsically easier to design than others. However, up to now, designability has been shown only in highly restrictive lattice models. Our calculations indicate that the qualitative features of designability in lattice models are also exhibited off-lattice. Namely, a small minority of off-lattice structures are distinguished by high designability: these structures are lowest-energy states for many more than their share of sequences. Moreover, the sequences associated with these structures have enhanced thermodynamic stability. The work presented here, using an off-lattice model for protein-backbone configurations, makes it more plausible that designability applies to real proteins. Of course, the model used in the current study is highly simplified—it is a low-resolution discrete model of short chain with a very simple potential function. There is still a long way to go to show the designability principle in real proteins.

Nonetheless, the insensitivity to model parameters of the results presented suggests that our highly designable structures are possible candidates for real protein design. It is therefore worthwhile to study some of our best candidates in detail and to understand what architectural properties distinguish the most designable structures from the least designable ones and how the most designable ones compare with known natural structures.

Representative configurations of some of the most designable structures are shown in Figure 1(a–c). A striking characteristic of the highly designable structures is that each has a well-defined core consisting of a small subset of the amino acids of the chain. For example, in Figure 5 we have plotted the inaccessible surface area of each amino acid along the chain for the configuration appearing in Figure 1(b). Observe that 5 of the 23 amino acids are more than 70% buried. Also shown in Figure 5 is the probability that a hydrophobic amino acid occupies a particular site, averaged over all HP-sequences that design the structure, revealing the preference of hydrophobic amino acids for the core.

A quantitative measure of the core in a structure is the variance v_s of the exposure vector \vec{A} : $v_s = (1/N) \sum_i \bar{a}_i^2 - (1/N^2) (\sum_i \bar{a}_i)^2$. In Figure 6, we plot v_s versus the designability N_s . On average the two quantities correlate well; however, the scatter of the data is large in the region of low

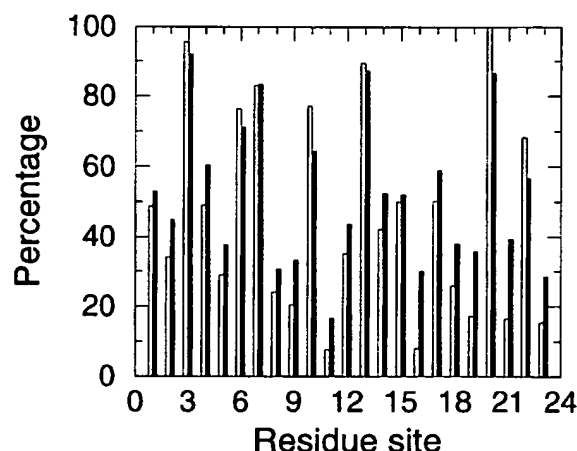


Fig. 5. Solid bars: Inaccessible surface for residues (C_β spheres) of the highly designable configuration shown in Figure 1(b). Hollow bars: Probability, averaged over all HP-sequences that design the configuration, that a particular site along the chain is occupied by a hydrophobic amino acid.

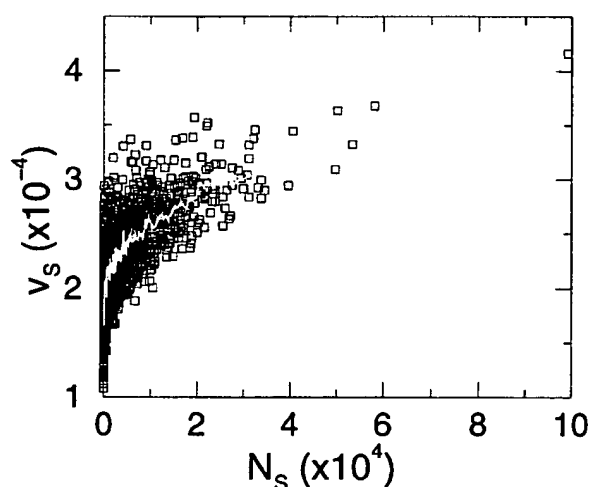


Fig. 6. The average variance v_s of a cluster against the designability N_s of the cluster for the 23-mer. The 5000 most compact configurations participate in the calculation, $\lambda = 0.4$ Å, and $r_\beta = 1.9$ Å. Gray line: running average with bin size 30.

N_s : structures with well-formed cores are not necessarily highly designable.

A zinc finger-like fold emerges from our calculation as one of the most designable structures. The fold [Fig. 1(b)] does not simply replicate 1PSV [Fig. 1(d)], on which we optimized our angle set. The structure of 1PSV is too open to be designable within our model because the small, uniformly sized side-chains cannot fill the large opening between the α -helix and the β - β turn in 1PSV. It is of interest that the model produces a highly designable solution by collapsing the α -helix onto the β - β turn.

Another of our most designable structures is similar to another small natural fold, the helix-turn-helix [see Fig. 1(c)]. Some of our most designable structures [e.g., that shown in Fig. 1(a)] do not resemble any known natural

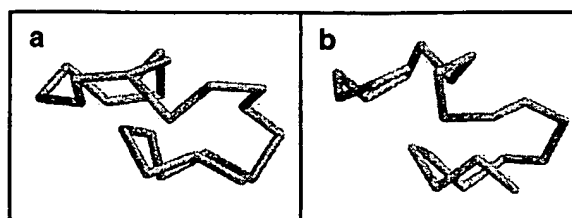


Fig. 7. a: Backbone configuration of the 11th most designable 23-mer structure, using untargated angle set (see text): $(\phi, \psi) = (-55^\circ, 135^\circ)$, $(-126^\circ, 145^\circ)$, and $(-85^\circ, -25^\circ)$, with a mean crms of 3.6 Å on a representative subset of natural structures segmented into subchains of 21 amino acids. For this calculation, the amino acids are represented by spheres of radius $r_a = 1.52$ Å centered on the C_α carbons only. b: Backbone configuration of the zinc finger 1NC8, truncated to 23 amino acids.²⁵

folds. These structures are candidates for the design of truly novel folds.

Targeting a fold by fitting the angle set to a single chosen structure is not essential. For example, we can obtain a suitable angle set by choosing two pairs of dihedral angles (ϕ, ψ) within the β -sheet region and one pair from the α -helix region, locally optimizing on 160 representative natural structures from the PDB database.²¹ Among the most designable structures emerging for this angle set is the zinc finger-like structure in Figure 7(a), shown next to its apparent natural counterpart, 1NC8 [Fig. 7(b)].²⁵

Recently, many studies have been conducted on the relation between the folding kinetics and the topology of native states.^{26–36} In particular, it has been shown that folding rates and the topology of the transition states are closely related to the topology of the native states. In other words, the native state topology, which in this context is often measured in terms of contact order,^{26,35} largely determines how a protein folds. It would be interesting to compare the two roles the native state topology plays: in folding kinetics and in the designability and thermodynamic stability. However, such a comparative study would preferably be done in systems of longer chains than used in the current study. Although it is tempting to think that there is a deep connection between the two roles of topology, one should note that there is a huge variation in folding rates among natural proteins,³³ which are presumably highly designable and thermodynamically stable. It appears that designability is largely governed by the surface-core patterning,¹⁵ whereas folding kinetics depends more on the ease of forming native contacts (the contact order).

In summary, we have computed the designabilities of structures within an off-lattice model of realistic protein-backbone configurations. Highly designable structures emerge with remarkable insensitivity to model parameters. The sequences that design these structures have strongly enhanced mutational stability and a large energy gap between the native fold and the lowest non-native conformation. In this light, it is interesting that recent mutation studies on some small proteins show that they maintain their native folds even when about half of their residues are replaced by alanine.^{37,38} Some of our highly

designable structures correspond closely to natural folds, such as the zinc finger and helix-turn-helix motifs. Others do not resemble existing structures and are candidates for ab initio design of novel protein folds.

REFERENCES

1. Beasley JR, Hecht MH. Protein design: the choice of de novo sequences. *J Biol Chem* 1997;272:2031–2034.
2. Baltzer L. Functionalization of designed folded polypeptides. *Curr Opin Struct Biol* 1998;8:466–470.
3. Cao AN, Lai LH, Tang YQ. The current state and prospect of de novo protein design. *Prog Biochem Biophys* 1998;25:197–201.
4. Giver L, Arnold FH. Combinatorial protein design by in vitro recombination. *Curr Opin Chem Biol* 1998;2:335–338.
5. Regan L, Wells J. Engineering and design: recent adventures in molecular design—editorial overview. *Curr Opin Struct Biol* 1998;8:441–442.
6. Schafmeister CE, Stroud RM. Helical protein design. *Curr Opin Biotechnol* 1998;9:350–353.
7. Shakhnovich EI. Protein design: a perspective from simple tractable models. *Fold Design* 1998;3:R45–R58.
8. DeGrado WF, Summa CM, Pavone V, Nastri F, Lombardi A. De novo design and structural characterization of proteins and metalloproteins. *Annu Rev Biochem* 1999;68:779–819.
9. Harbury PB, Plecs JJ, Tidor B, Alber T, Kim PS. High-resolution protein design with backbone freedom. *Science* 1998;282:1462–1467.
10. Struthers MD, Cheng RP, Imperiali B. Design of a monomeric 23-residue polypeptide with defined tertiary structure. *Science* 1996;271:342–345.
11. Dahiyat BI, Mayo SL. De novo protein design: fully automated sequence selection. *Science* 1997;278:82–87.
12. Dahiyat BI, Sarisky CA, Mayo SL. De novo protein design: towards fully automated sequence selection. *J Mol Biol* 1997;273:789–796.
13. Chothia C. One thousand families for the molecular biologist. *Nature* 1992;357:543–544.
14. Li H, Helling R, Tang C, Wingreen N. Emergence of preferred structures in a simple model of protein folding. *Science* 1996;273:666–669.
15. Li H, Tang C, Wingreen NS. Are protein folds atypical? *Proc Natl Acad Sci USA* 1998;95:4987–4990.
16. Govindarajan S, Goldstein RA. Searching for foldable protein structures using optimized energy functions. *Biopolymers* 1995;36:43–51.
17. Govindarajan S, Goldstein RA. Why are some protein structures so common? *Proc Natl Acad Sci USA* 1996;93:3341–3345.
18. Finkelstein AV, Ptitsyn OB. Why do globular proteins fit the limited set of folding patterns? *Prog Biophys Mol Biol* 1987;50:171–190.
19. Yue K, Dill KA. Forces of tertiary structural organization in globular proteins. *Proc Natl Acad Sci USA* 1995;92:146–150.
20. Mélin R, Li H, Wingreen NS, Tang C. Designability, thermodynamic stability, and dynamics in protein folding: a lattice model study. *J Chem Phys* 1999;110:1252–1262.
21. Park BH, Levitt M. The complexity and accuracy of discrete state models of protein structure. *J Mol Biol* 1995;249:493–507.
22. Flower DR. SERF: A program for accessible surface area calculations. *J Mol Graph Model* 1997;15:238–244.
23. Creighton TE. *Proteins*. New York: Freeman; 1993. p160–162, 236–237.
24. Lau KF, Dill KA. Lattice statistical mechanics model of the conformational and sequence spaces of proteins. *Macromolecules* 1989;22:3986–3997.
25. Kodera Y, Sato K, Tsukahara T, Komatsu H, Maeda T, Kohno T. High-resolution solution NMR structure of the minimal active domain of the human immunodeficiency virus type-2 nucleocapsid protein. *Biochemistry* 1998;37:17704–17713.
26. Plaxco KW, Simons KT, Baker D. Contact order, transition state placement and the refolding rates of single domain proteins. *J Mol Biol* 1998;277:985–994.
27. Chan HS. Protein folding: matching speed and locality. *Nature* 1998;392:761–763.
28. Portman JJ, Takada S, Wolynes PG. Variational theory for site resolved protein folding free energy surfaces. *Phys Rev Lett* 1998;81:5237–5240.
29. Goldenberg DP. Finding the right fold. *Nat Struct Biol* 1999;6:987–990.
30. Alm E, Baker D. Matching theory and experiment in protein folding. *Curr Opin Struct Biol* 1999;9:189–196.
31. Fersht AR. Transition-state structure as a unifying basis in protein-folding mechanisms: contact order, chain topology, stability, and the extended nucleus mechanism. *Proc Natl Acad Sci USA* 2000;97:1525–1529.
32. Maritan A, Micheletti C, Banavar JR. Role of secondary Motifs in fast folding polymers: a dynamical variational principle. *Phys Rev Lett* 2000;84:3009–3012.
33. Baker D. A surprising simplicity to protein folding. *Nature* 2000;405:39–42.
34. Clementi C, Nymeyerson Onuchic JN. Topological and energetic factors: what determines the structural details of the transition state ensemble and “en-route” intermediates for protein folding? An investigation for small globular proteins. *J Mol Biol* 2000;298:937–953.
35. Plaxco KW, Simons KT, Ruczinski I, Baker D. Sequence, stability, topology and length; the determinants of two-state protein folding kinetics. *Biochemistry* 2000;39:11177–11183.
36. Guerois R, Serrano L. Protein design based on folding models. *Curr Opin Struct Biol* 2001;11:101–106.
37. Kuroda Y, Kim PS. Folding of bovine pancreatic trypsin inhibitor (BPTI) variants in which almost half the residues are alanine. *J Mol Biol* 2000;298:493–501.
38. Brown BM, Sauer RT. Tolerance of Arc repressor to multiple-alanine substitutions. *Proc Natl Acad Sci USA* 1999;96:1983–1988.